Queen Margaret University

EDINBURGH

School of Health Sciences

Dietetics, Nutrition & Biological Sciences

Final Report to the Scottish Government
Rural and Environment Research and Analysis Directorate (RERAD)

Dr Iain F Gow

"Pharmacological & Physiological Characterisation of the Rat Mammary Artery at Different Stages of Reproduction"

1st April, 2006 – 31st March, 2009

hannah research

TRANSITIONAL FUNDING GRANT - FINAL REPORT

Dr Iain F Gow

1) RESEARCH ACTIVITY INFORMATION

a) Title of Research Activity: "Pharmacological & Physiological Characterisation of the Rat Mammary Artery at Different Stages of Reproduction"

b) Principal Investigator: Dr Iain F Gow

c) Named Investigators: Mr David Blatchford (1st April 2006 – 31st December 2007), and Dr Duncan Macgregor (1st January 2008 – 31st March 2009)

d) Reporting period: 1st April 2006 – 31st March 2009

2) EXECUTIVE SUMMARY

a) Summary of Research Activity:

The overarching-aim of the project was to assess the responsiveness of the rat mammary artery during different stages of the reproductive cycle. This has implications for not only normal physiology, but also heart disease, since women who undergo coronary artery bypass surgery using mammary arteries as the conduit and who have breastfed their offspring have a significantly better prognosis that those who have never breastfed.

The first major achievement of the project was the establishment of a breeding colony of rats. This took much longer than expected since initially, the rate of pregnancy was very low, and due to the need for randomisation, the optimum colony mass was not achieved until several months after arriving at Strathclyde. However having overcome the complexity of organising such a colony, we are now very well placed to perform other studies where randomised states of reproduction are required.

Experimental results showed that the artery preparations were much more variable than other similar vessels, possibly because of a high intrinsic tone. This variability meant more observations (up to 19 in some instances) were required to be able to detect significant differences (if present). Preliminary experiments showed that the arteries responded to noradrenaline and serotonin, but bearing in mind the increased numbers of observations required, we concentrated in assessing responses mediated by noradrenergic receptors. Use of contractile agents relatively specific for certain receptors indicated that contract was mediated by alpha, most likely predominantly $alpha_{1/1A}$ receptors since drugs acting at those receptors caused contraction at all stages of the reproductive cycle.

Arteries from animals undergoing the reproductive cycle contracted more strongly in the presence of known contractile agents than the controls. Conversely, timed-control animals from the lactation period showed relaxation with an $alpha_2$ agonist (UK 14,304) whereas the reproducing animals did not. The artery luminal area was greater in lactating animals than controls, and more muscle was present in pregnant/lactating RMAs than controls.

We demonstrated that single vascular smooth muscle cells could be isolated from the rat mammary artery. Most of the proposed work was completed, with modifications to the project being made to take into account the changes in personnel. We conclude that functional changes do occur in the RMA during the reproductive cycle, and these are accompanied by structural changes of the vessel. Vessels from animals post-weaning contracted more strongly than those from controls, suggesting reproduction does produce changes in vascular responsiveness which are retained long after lactation has ceased.

However there is a paradox requiring further investigation which is that at a time when most muscle is present (lactation), the contractile force generated appears to be at its lowest.

b) Summarised Main Objectives:

i) Characterisation of the receptor profile of the Rat Mammary Artery (RMA)
ii) Assessment of mechanisms of differences in the RMA at different stages of the reproductive cycle (virgin, pregnant, peak lactation, and post-weaning).
iii) Determine changes in the RMA following treatment of non-pregnant female rats with synthetic oestrogen and progesterone to mimic pregnancy

3) INTRODUCTION TO THE RESEARCH ACTIVITY AND BACKGROUND INFORMATION

a) Overall aims of the Research Activity, background and objectives: This project proposed to study the behaviour of the rat mammary artery (RMA) model, to assess changes occurring during the reproductive cycle. In breast cancer, it is known that a single pregnancy and breastfeeding protects the mother against the disease to a certain extent, though the mechanism of this remains unknown. Permanent changes occur in the blood vessels of the breast when women have breastfed their offspring, and this could result in an increased blood and oxygen supply to the gland, and faster removal of toxins, both of which may be protective against tumour establishment or proliferation. By characterising the behaviour of the RMA more fully at different stages of the reproductive cycle, we will be able to see if any observed changes are retained once milk production has stopped. One of the mechanisms put forward to explain these changes has been that exposure of the mammary gland to the levels of reproductive hormones found in pregnancy confers protection on the gland; the supported project sought to examine the vascular responses in the mammary gland under controlled conditions of reproduction. The major objectives where thus to examine the mammary artery responsiveness during the reproductive cycle, and seek evidence that changes occurred were retained after weaning.

b) Staff employed on the project:

i) Dr Iain F Gow (1st April 2006 – 30th November 2007)
ii) Mr David Blatchford (1st April 2006 – 31st December 2007)
iii) Dr Duncan Macgregor (1st January 2008 – 31st March 2009)

c) Timetable and milestones as in original proposal: the original proposal did not include a timetable or milestones

d) Any major changes in the Research Activity: due to the loss of a senior researcher from the project (IFG) and the changeover in research associate (DB to DMcG), we could not perform the hormonal implant, receptor identification, or ion-flux studies in this project. However the information gathered from the SEERAD-funded project has enabled us to submit a strong application to the BBSRC to pursue areas not addressed (see Annex 10). We chose therefore to concentrate on the functional measurements combined with histological data assessing any physical changes in the artery which might account for this.

4) RESULTS - Vessel contractions/relaxations obtained using noradrenaline (NA – alpha/beta agonist), phenylephrine (PE – $alpha_1$ agonist), A61603 ($alpha_{1A}$ agonist), and UK 14, 304 ($alpha_2$ agonist) were measured by standard wire myography of isolated mammary vessels obtained from female rats. Rat were selected from a colony established in such a way that animals (and age and sex-matched virgin controls – AMVCs) at different stages of the

reproductive cycle were available in a statistically random manner. The stages used were Pregnant (17 days from a mating at age seven weeks), Lactating (30 days after mating, 10-12 after parturition), and Weaned (60 days after mating, 30 days after pup removal). Data for myography were analysed by Graphpad Prism, groups contained between 8-20 animals. For histology, isolated RMAs were fixed using 10% formaldehyde perfused at 100 mmHg to maintain the architecture of the vessel at *in vivo* pressures. The arteries were then embedded in wax and stained with haematoxylin and eosin. Cross-sectional images were captured using a digital camera, and measurements made using Motic Image Plus 2.0 software. Isolated vascular smooth muscle cells were prepared from RMA by gentle digestion with papain/collagenase. Data were analysed with ANOVA (one- or two-way as appropriate) followed by the Student's *t* test with Bonferroni *post-hoc* modification or standard *t* test as appropriate. Data are presented in Annexes for reference. The results overall indicate that functional changes occur in the rat mammary artery during the reproductive cycle, and that changes persist well after reproduction has ceased. As far as we are aware, this is the first demonstration of such an effect in major mammary arteries, and is consistent with the hypothesis that pregnancy and lactation create permanent changes in breast physiology. These results are particularly exciting since they provide an excellent foundation from which to build and seek further funding to progress this extremely important area. The project involved a great deal of assistance from and co-ordination with the university biomedical planning unit, biophotonics, and the cardiovascular research group. These well-established groups assisted greatly in ensuring the project started as quickly as possible, and that resources were available for the research. In summary, major findings/outcomes were:

- A colony capable of regularly providing animals at various stages of reproduction in a statistically random manner was established
- RMAs responded in a dose-dependent manner to all the above agonists, contracting with most, but relaxing with UK 14, 304 (Annexes 1,2)
- There was no change in sensitivity to the contractile agents, but the controls showed a significant increase in sensitivity to UK 14, 304 at around day 30 as measured by the pEC_{50} (Annexes 3-5) .
- RMA contractions with NA, A61603, and PE were all greater in the reproducing animals than the AMVCs (Annex 6)
- RMA contractions with NA and PE were greater in weaned animals than AMVCs (Annex 6)
- RMA contractions with PE were greater in pregnant animals than AMVCs (Annex 6)
- RMA contractions with PE were greater in weaned compared with lactating rats (Annex 6)
- RMA relaxations were greater in AMVCs at day 30 compared with day 17 (Annex 6)
- the RMA luminal area in lactating rats is greater than pregnant, weaned, or AMVCs of any age (Annexes 7 - 8)
- the cross-sectional area of muscle in RMAs from pregnant and lactating animals is greater than the corresponding AMVCs (Annexes 7 - 8)
- the muscle:lumen area ratio is less in lactating animals than the AMVC (Annexes 7 - 8)
- viable vascular smooth muscle cells could be isolated from RMA by gentle digestion with papain/collagenase (Annex 9)

There are a great many benefits arising from the transitional funding: it has allowed us to consolidate and further develop an unique model first established at the Hannah. We have made significant progress in an area of research which we believe is not only extremely important from the biological stance, but retains the spirit, philosophy and direction of work from the Hannah Institute. The period of Transitional Funding has allowed two seniors researchers to gain substantive posts, and another to gain valuable experience in a range of cardiovascular

techniques. In addition, we managed to provide experience and training for both undergraduate and postgraduate students during this time. Finally, and possibly most importantly, it has provided substantial data, some of which we have already used (Annex 10) in seeking other funding to continue and expand the research.

5) CONCLUSIONS

a) Main findings:

The main findings indicate that physiological changes occur in the mammary gland blood vessels during reproduction, and the single cycle of reproduction seen here is enough to produce changes which persist for at least several months. This is significant when it is considered that unknown changes persist in human and other animal mammary glands following a single pregnancy which confers a degree of protection against breast cancer. To date, the role of the blood supply has largely been ignored, attention being devoted almost exclusively to the mammary epithelial cell. The results from this study shows that vascular changes may also play a role.

There are several unanswered questions from the study: although functional changes have been observed, why is the strength of contraction weakest when the muscle mass is apparently greatest (i.e. peak lactation)? Are there changes in receptor numbers/characteristics which could explain this? The AMVCs were not expected to show differences between groups, yet the animals sacrificed on Day 30 were more sensitive to UK 14, 304 than at the other time points. This would not be due to seasonal/temporal effects since the culls were randomised throughout the year. The AMVC contractions paralleled (but were always less than) the profiles of the reproducing animals. Again, due to the randomisation of sampling, we feel able to exclude temporal effects. It is particularly exciting that a study which we believe to be designed reasonably robustly has thrown up challenges which remain to be addressed by further studies.

b) Consequences of findings from the Research Activity as a whole:

The overall consequences of the Research Activity is that clearly reproduction has a significant, persistent effect on mammary artery tone. Muscle mass increases, possibly through muscle cell hypertrophy or proliferation, but then decreases but with an increase in the force generated during contraction in response to $alpha_1$ agonists. Changes in size and blood flow in mammary arteries in women who have breastfed have been reported, and a history of breastfeeding has been associated with an improved prognosis in women undergoing coronary artery bypass using internal mammary arteries as conduits. Thus it is clear that the functional changes of the mammary artery need to be investigated further, since a better understanding of the normal physiology of this vessel may have important applications in diseases such breast cancer or coronary heart disease.

6) COMMUNICATED OUTPUTS

a) Refereed Publications: "Blatchford, D & Gow, IF. Contraction induced by noradrenaline or serotonin is increased in the rat superficial epigastric artery during lactation." From the University of Oxford, Winter 2006 Meeting: Proceedings of the British Pharmacological Society http://www.pa2online.org/abstracts/Vol4Issue2abst137P.pdf

b) Popular and trade articles: none

c) Presentations at scientific meetings: "Blatchford, D & Gow, IF. Contraction induced by noradrenaline or serotonin is increased in the rat superficial epigastric artery during lactation." British Pharmacological Society, University of Oxford, Winter 2006

d) Other reports/publications/communications: preliminary data used in BBSRC grant application

e) Communication of research to the public: none

f) Technology Transfer: none

g) Patents applied for: none

7) RESOURCES

a) To follow, as per email from RERAD to University of Strathclyde dated the 17th February

b) Investigators funded by this project, and related studentships:

i) Dr Iain F Gow (Staff: 1st April 2006 – 30th November 2007)

ii) Mr David Blatchford (Staff: 1st April 2006 – 31st December 2007)

iii) Dr Duncan Macgregor (Staff:1st January 2008 – 31st March 2009)

iv) Two third-year pharmacology students from Strathclyde (Ms Lisa Michel and Mr Peter Gordon) spent six weeks working in the laboratory at Strathclyde (June-July 2007) on studies related to this project. This stimulated their interest in research so much they are both now doing PhD degrees.

v) An MRes student (Mr Ahmad Alsaadi) worked on some of the histological measurements, for which due acknowledgement is given.

c) Other staff contributing to this project: none

d) Destination of Principal Investigator and Investigators after the end of the Transitional Funding Grant:

i) Dr Iain F Gow – indefinite contract as a Lecturer at Queen Margaret University, Edinburgh, from 1st December 2007

ii) Mr David Blatchford – five-year contract as Biophotonics Technician, University of Strathclyde, from 1st December 2007

iii) Dr Duncan Macgregor – awaiting outcome of BBSRC grant application submitted by Dr Gow, otherwise contract ends 31st March 2009

8) ACKNOWLEDGEMENTS

a) We acknowledge with great gratitude the assistance of RERAD/SEERAD in funding the project, and in particular the role of RERAD and other Ministers of the Scottish Government, and other Ayrshire MSPs in allowing flexibility in the original Terms and Conditions of the Grant in the case of Dr Gow (Principle Investigator, PI) who left to take up another post at Queen Margaret University, Edinburgh. This flexibility had several benefits:

i) savings were made since although the PI had transferred to another organisation's funding, a significant proportion of the project was completed which would otherwise have been lost

ii) it assisted cross-institutional collaboration

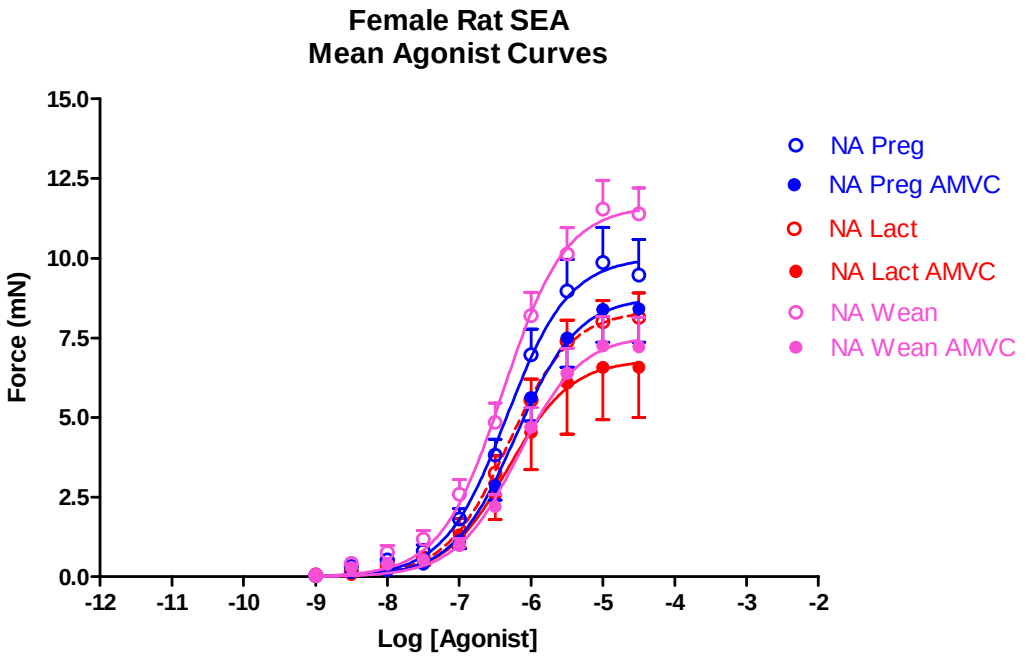
iii) the project produced results which will assist in attracting other funding to continue Hannah science in Scotland

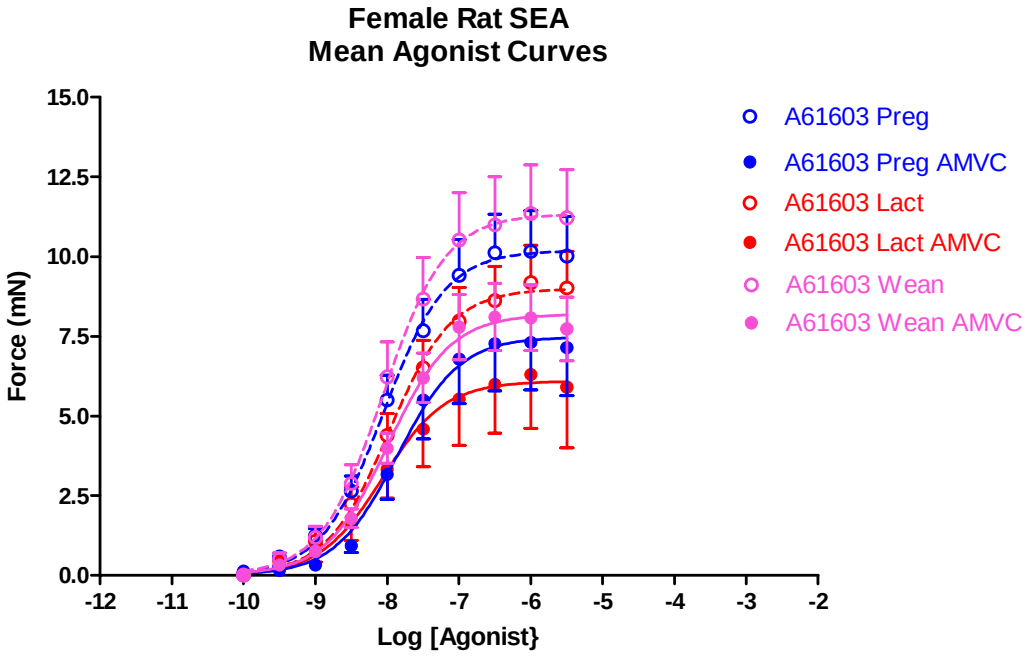
iv) support staff jobs were not lost since the project was active until the agreed date

v) the agreement of Ministers to allow transfer of Dr Gow's Transitional Funding Equipment means that Queen Margaret University has an excellent foundation from which to develop its biomedical research strategies

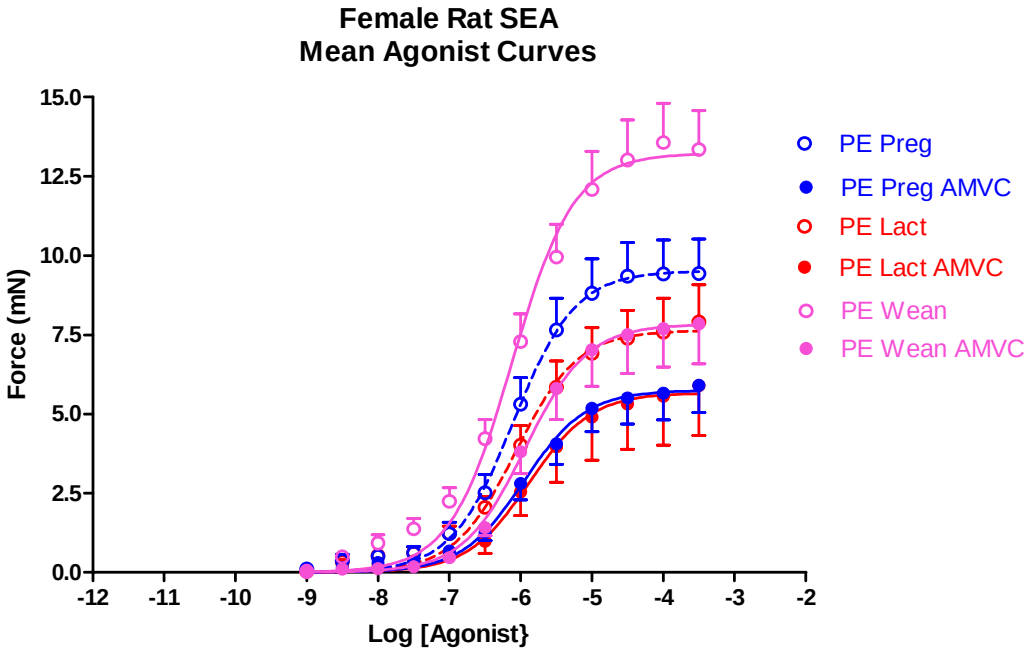
9) ANNEXES

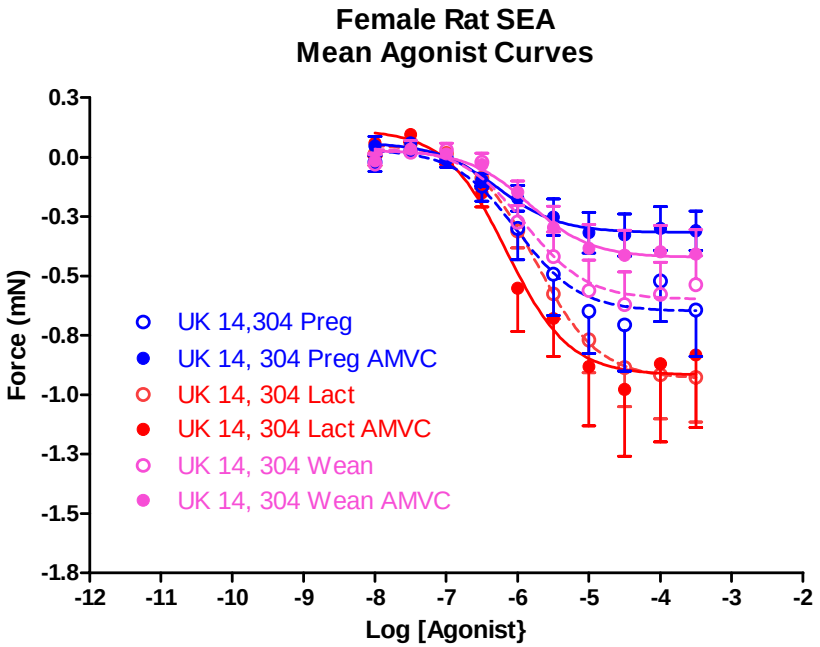
a) Annex 1:



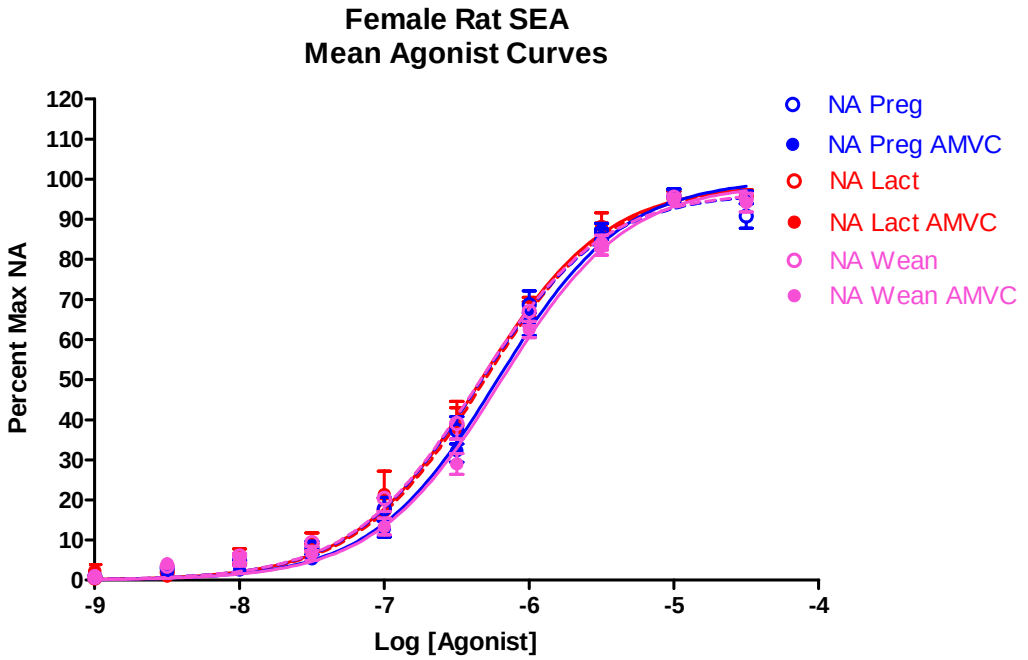


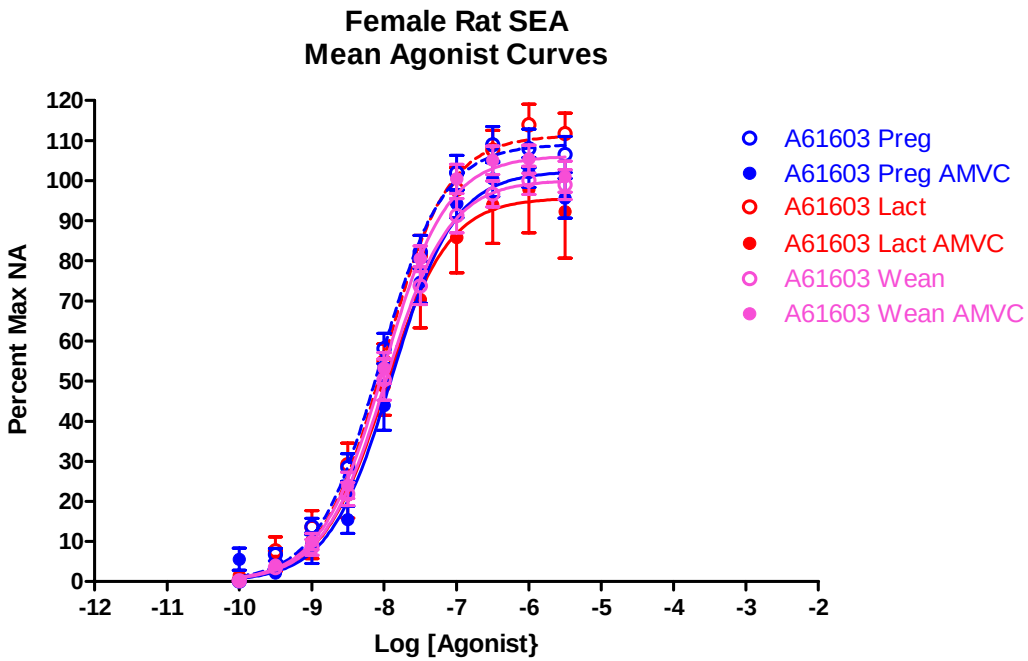
b) Annex 2:


Female Rat SEA
Mean Agonist Curves
Force (mN)
15.0
12.5
10.0
7.5
5.0
2.5
0.0
-12
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
Log [Agonist}
PE Preg
PE Preg AMVC
PE Lact
PE Lact AMVC
PE Wean
PE Wean AMVC

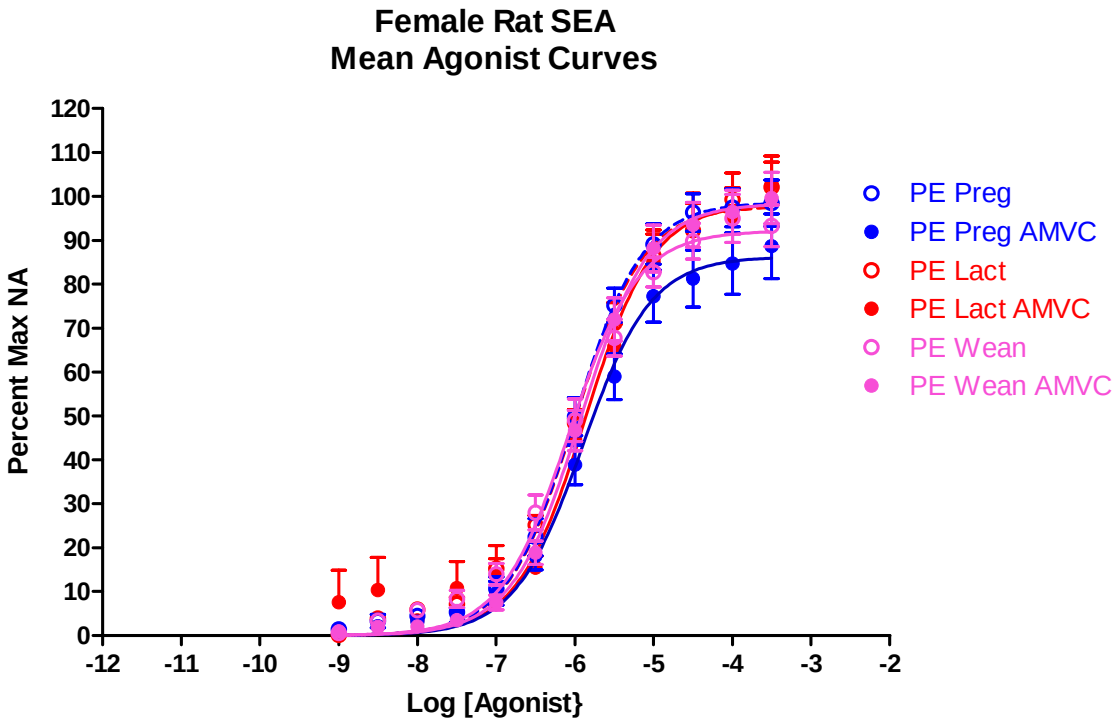

Female Rat SEA
Mean Agonist Curves
Force (mN)
0.3
0.0
-0.3
-0.5
-0.8
-1.0
-1.3
-1.5
-1.8
-12
-11
-10
-9
-8
-7
-6
-5
-4
-3
-2
Log [Agonist}
UK 14,304 Preg
UK 14, 304 Preg AMVC
UK 14, 304 Lact
UK 14, 304 Lact AMVC
UK 14, 304 Wean
UK 14, 304 Wean AMVC

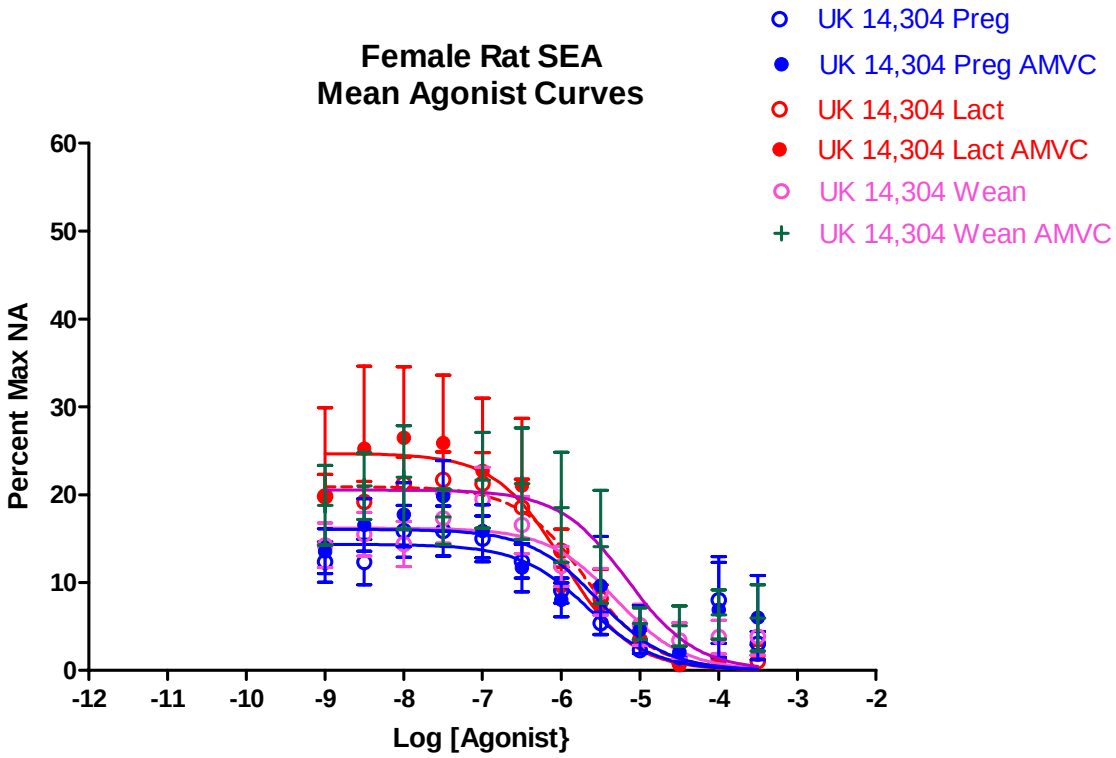
c) Annex 3:


Female Rat SEA
Mean Agonist Curves
Percent Max NA
Log [Agonist]
NA Preg
NA Preg AMVC
NA Lact
NA Lact AMVC
NA Wean
NA Wean AMVC

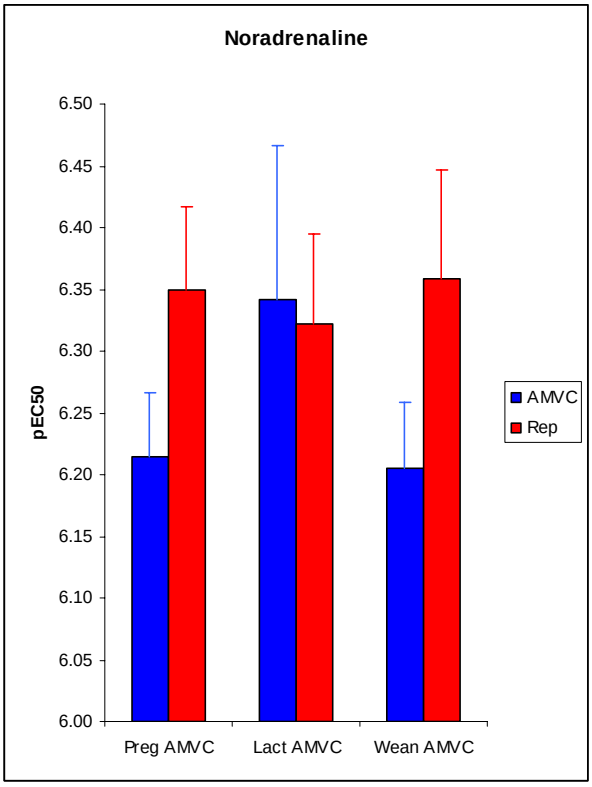

Female Rat SEA
Mean Agonist Curves
Percent Max NA
Log [Agonist}
A61603 Preg
A61603 Preg AMVC
A61603 Lact
A61603 Lact AMVC
A61603 Wean
A61603 Wean AMVC

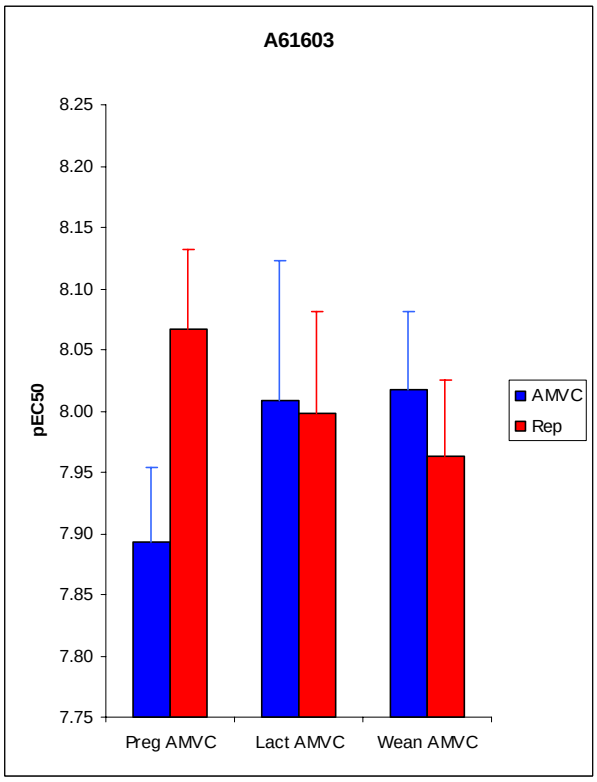
d) Annex 4

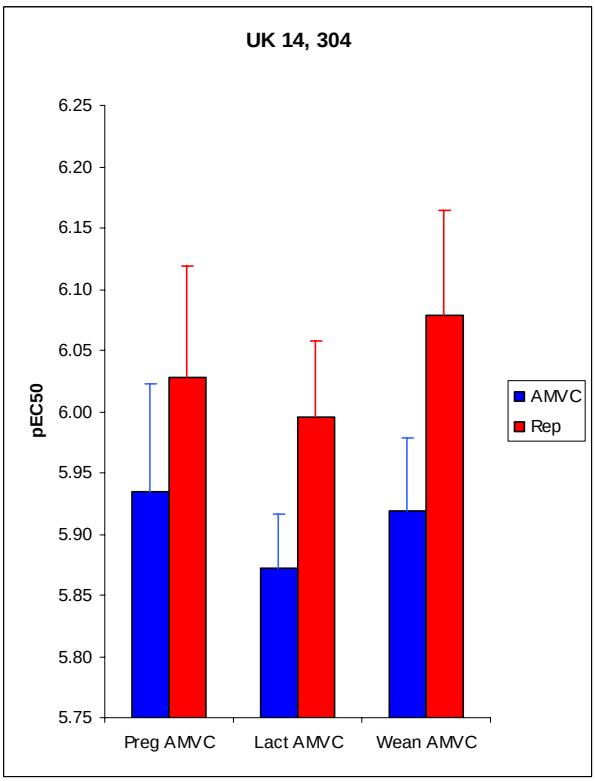

Female Rat SEA
Mean Agonist Curves
Percent Max NA
Log [Agonist}
PE Preg
PE Preg AMVC
PE Lact
PE Lact AMVC
PE Wean
PE Wean AMVC

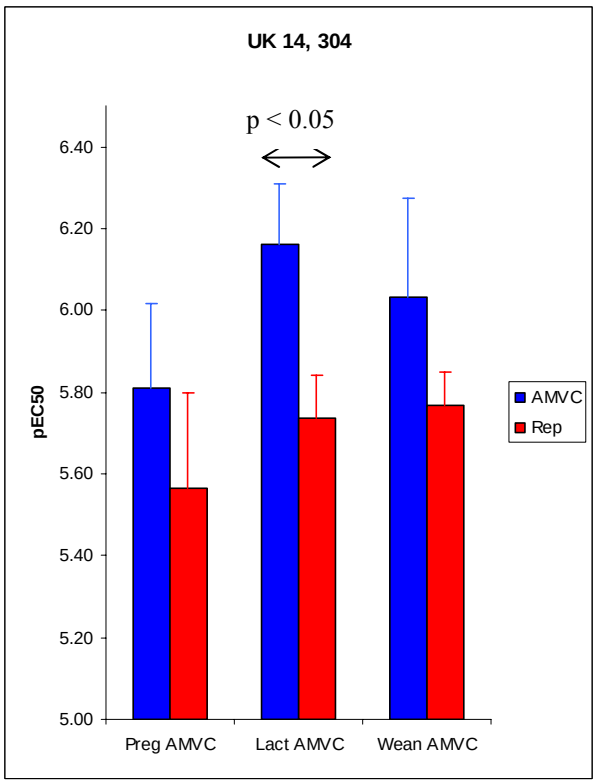

Female Rat SEA
Mean Agonist Curves
Percent Max NA
Log [Agonist}
UK 14,304 Preg
UK 14,304 Preg AMVC
UK 14,304 Lact
UK 14,304 Lact AMVC
UK 14,304 Wean
UK 14,304 Wean AMVC

e) Annex 5:

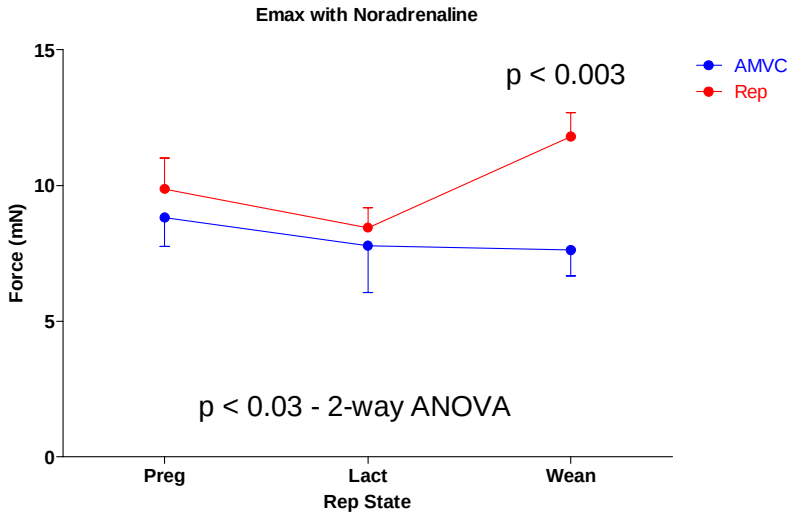

Noradrenaline
pEC50
6.50
6.45
6.40
6.35
6.30
6.25
6.20
6.15
6.10
6.05
6.00
Preg AMVC
Lact AMVC
Wean AMVC
AMVC
Rep

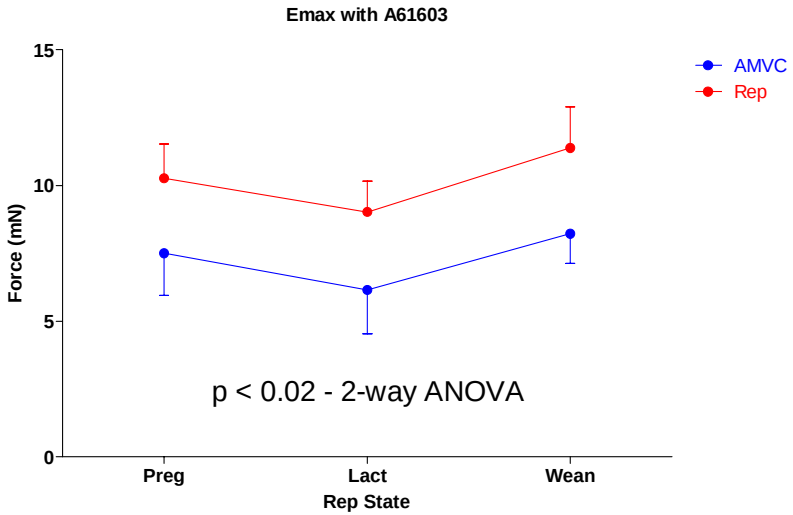

A61603
pEC50
8.25
8.20
8.15
8.10
8.05
8.00
7.95
7.90
7.85
7.80
7.75
Preg AMVC
Lact AMVC
Wean AMVC
AMVC
Rep

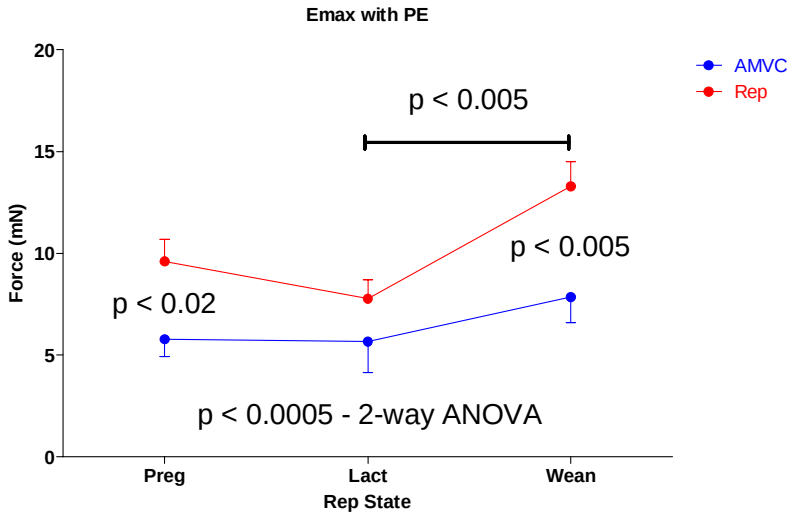

UK 14, 304
pEC50
6.25
6.20
6.15
6.10
6.05
6.00
5.95
5.90
5.85
5.80
5.75
Preg AMVC
Lact AMVC
Wean AMVC
AMVC
Rep

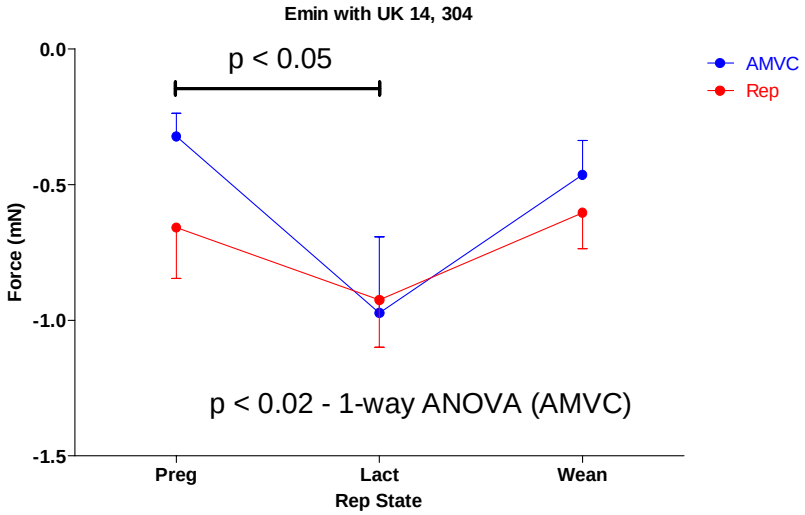

UK 14, 304
p < 0.05
pEC50
6.40
6.20
6.00
5.80
5.60
5.40
5.20
5.00
Preg AMVC
Lact AMVC
Wean AMVC
AMVC
Rep

f) Annex 6:

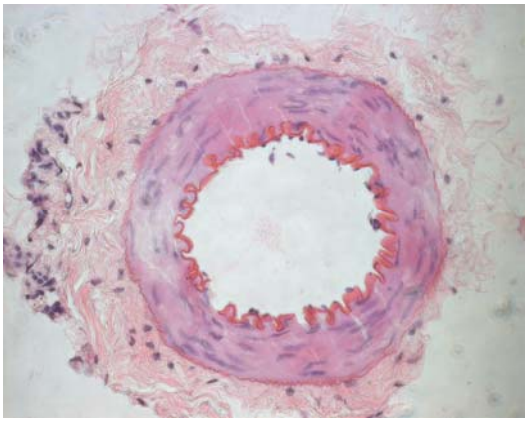

Emax with Noradrenaline
p < 0.003
p < 0.03 - 2-way ANOVA
Force (mN)
Rep State
Preg
Lact
Wean
AMVC
Rep


Emax with A61603
p < 0.02 - 2-way ANOVA
Force (mN)
Rep State
Preg
Lact
Wean
AMVC
Rep

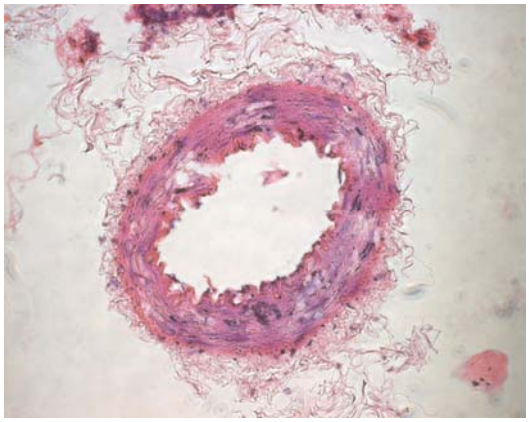

Emax with PE
p < 0.005
p < 0.005
p < 0.02
p < 0.0005 - 2-way ANOVA
Force (mN)
Rep State
Preg
Lact
Wean
AMVC
Rep


Emin with UK 14, 304
p < 0.05
p < 0.02 - 1-way ANOVA (AMVC)
Force (mN)
Rep State
Preg
Lact
Wean
AMVC
Rep

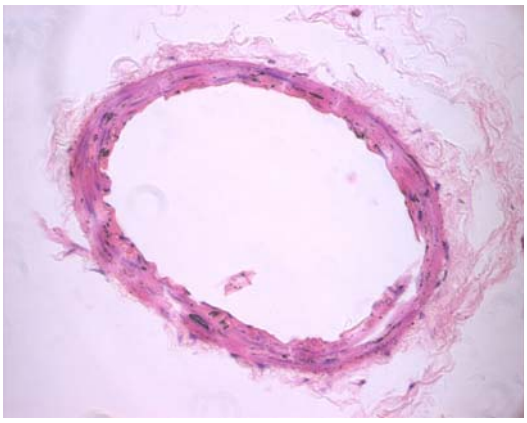
g) Annex 7



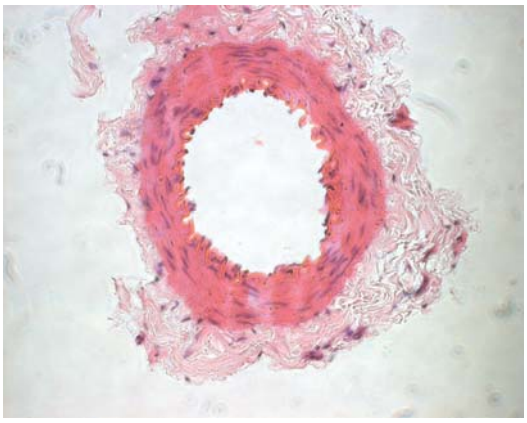
Pregnant



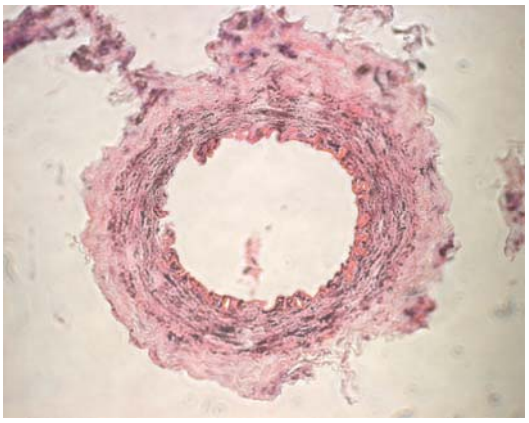
Pregnant AMVC



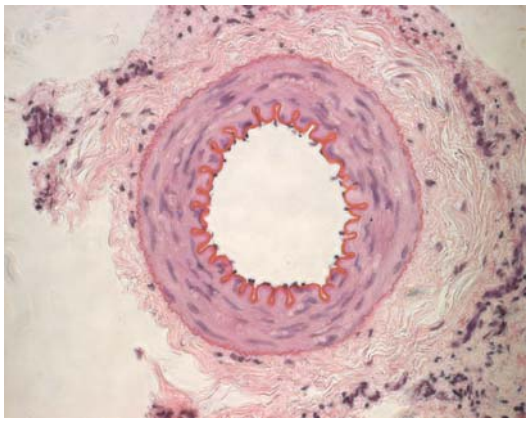
Lactating



Lactating AMVC

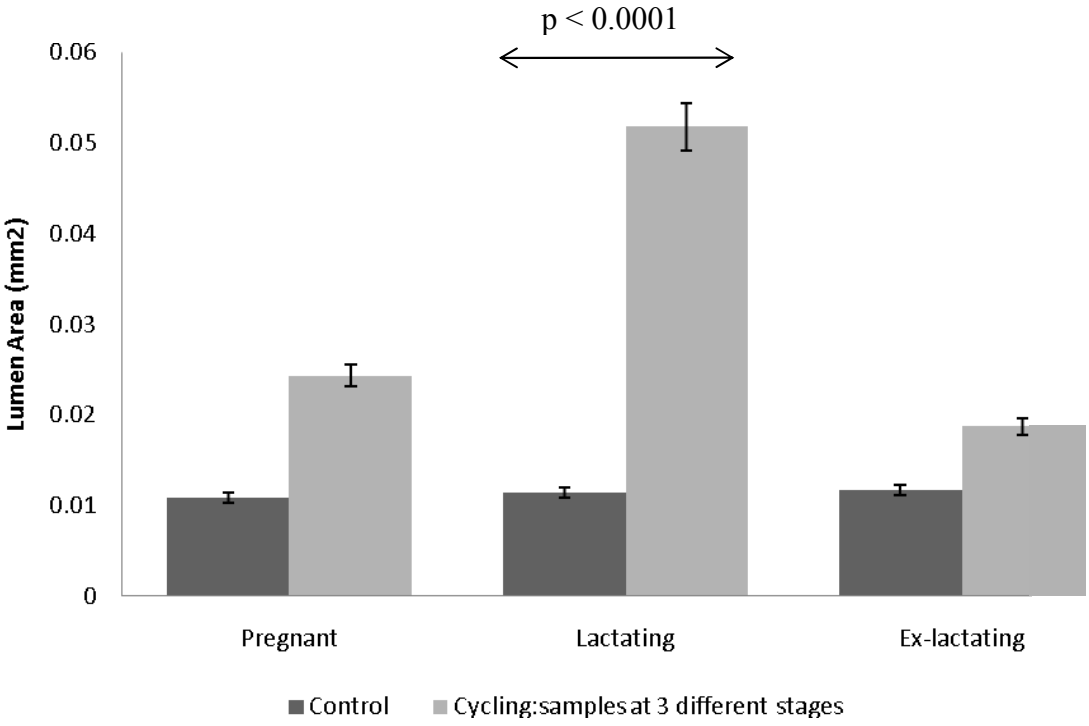


Weaned



Weaned AMVC

h) Annex 8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0.06
0.05
0.04
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0.02
0.01
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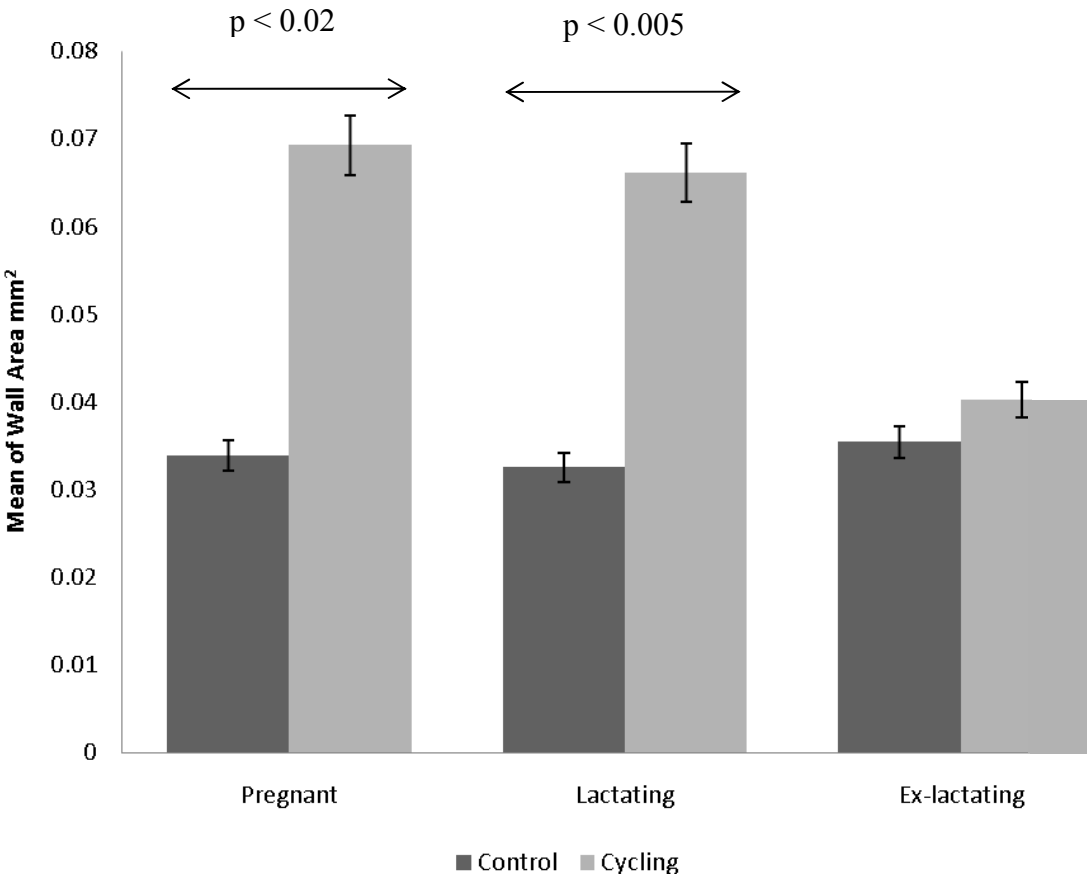
Lumen Area (mm2)
Pregnant
Lactating
Ex-lactating
Control
Cycling:samples at 3 different stages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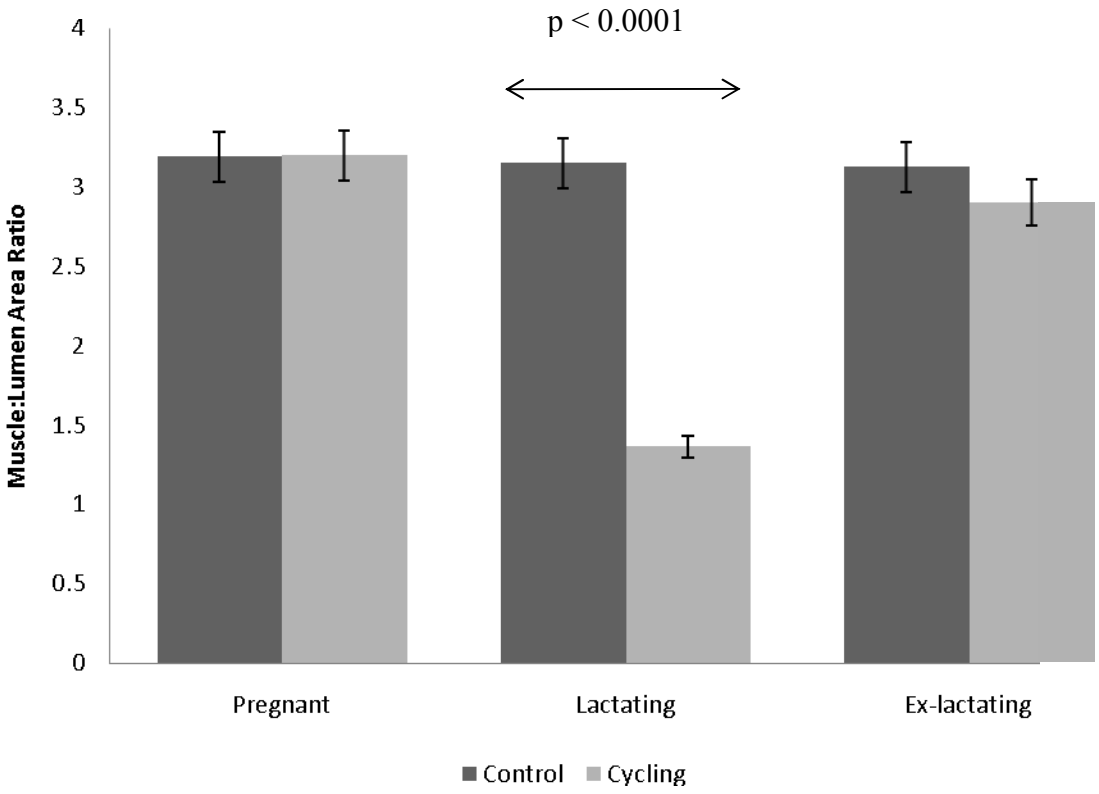
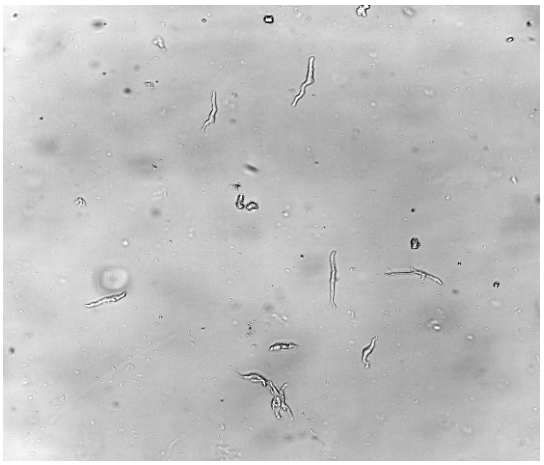
Mean of Wall Area mm2
Pregnant
Lactating
Ex-lactating
Control
Cycling



i) Annex 9



Isolated RMA vascular smooth muscle cells prepared by papain/collagenase digestion

BBSRC
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United Kingdom SN2 1UH
Telephone +44 (0) 1793 413200
Web http://www.bbsrc.ac.uk/

COMPLIANCE WITH THE DATA PROTECTION ACT 1998

In accordance with the Data Protection Act 1998, the personal data provided on this form will be processed by BBSRC, and may be held on computerised database and/or manual files. Further details may be found in the **guidance notes**

Standard PROPOSAL

Document Status: With Owner
BBSRC Reference:

Responsive mode - closing date 15 October 2008
Animal Sciences (AS)

Organisation where the Grant would be held (mandatory)

Organisation	Queen Margaret University	Research Organisation Reference:	RMA E2 project QMU
Division or Department	Dietetics Nutrition and Biological Sci		

Project Title (mandatory) [up to 150 chars]

The Role of Oestradiol in Structural and Functional changes in the Rat Mammary Artery during the Reproductive Cycle

Start Date and Duration (mandatory)

a. Proposed start date	01 April 2009	b. Duration of the grant (months)	24

Applicants (mandatory)

Role	Name	Organisation	Division or Department	How many hours a week will the investigator work on the project?
Principal Investigator	Dr Iain Gow	Queen Margaret University Edinburgh	Dietetics Nutrition and Biological Sci	3.75

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Objectives (mandatory)

List the main objectives of the proposed research in order of priority [up to 4000 chars]

Studies for the proposed project will use rodents undergoing normal reproduction as well as ovariectomised (rat/mouse) and oestrogen-receptor knock-out (mouse) models to allow control of circulating oestradiol and its targets. Ovariectomy followed by implantation of slow-release oestradiol (the major oestrogen in rodents) pellets will be used to replace the hormone levels appropriate for the stage of reproduction (i.e. late-pregnant, peak lacatation and weaned). Expertise and methodologies for the proposed experiments are well-established in the cardiovascular and biophotonics laboratories at the Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS).

Objective 1 - Oestradiol and Mammary Artery Remodelling:
A major objective is to investigate the degree and mechanisms of oestradiol-induced vascular remodelling of the mammary artery in the above groups by using proliferation assays applied to isolated vascular smooth muscle cells, and also with consideration of the distribution of specific vascular cell types measured by conventional histology, fluorescence immunohistochemistry, and dual-photon confocal microscopy.

Objective 2 - Oestradiol and Vascular Smooth Muscle Cell (VSMC) Ion Regulation:
We shall assess the interaction between endogenous oestradiol levels and Na+/H+ exchange activity in vascular smooth muscle cells isolated from the rodent mammary artery from the groups above. This involves the use of ion-sensitive fluorescent probes such as 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein (BCECF) or fura-2 for H+ and Ca2+ measurement respectively, techniques we have used successfully in the past to measure free ions in isolated cardiac or mammary epithelial cells.

Objective 3 -Oestradiol-induced Vascular Remodelling and Insulin-like Growth Factor
The levels of IGF-1 and IGF-1 receptor (IGF-1R) will be assessed in the smooth muscle layer of mammary arteries excised from the different treatment groups by fluorescent immunohistochemistry to determine whether or not they change when the animals experience different levels of oestradiol in vivo.

Objective 4 -Oestradiol and Mammary Artery Tone:
This objective will determine the effect in terms of oestradiol levels on function of the rodent (i.e. rat or mouse) mammary artery by measuring changes in vascular tone in mammary arteries in response to classical alpha adrenoceptor agonists. This will be achieved in the above groups by standard wire myograph techniques we have already used in developing the rodent mammary artery model.

Summary (mandatory)

Describe the proposed research in simple terms in a way that could be publicised to a general audience [up to 4000 chars]

The mammary gland is a dynamic organ, varying with age, menstrual cycle and reproductive state; it develops in the associated fat pad around puberty, and undergoes massive development during pregnancy, preparing the gland for milk production during lactation. Blood flow to the mammary gland is important since mammary blood flow increases around and after parturition/lactation, with 16% or 12% of cardiac output being directed to the gland in cows or rodents respectively at peak lactation; mammary arteries adapt to this by increasing artery muscle thickness during lactation in rats, though whether this is due to an increase in cell number or size is unknown. In addition, little is known about the intrinsic ability of mammary arteries to regulate blood flow, and there is even less information about their functional changes during the reproductive cycle, when the gland undergoes major anatomical and physiological changes. Conversely, other vessels associated with the reproductive system such as uterine blood vessels have been well-studied, and are known to be desensitised to agonists during pregnancy. Although studying the way reproduction changes mammary artery sensitivity and structure is interesting per se, there are also health-related benefits: in the UK, heart disease is still a major cause of mortality and morbidity, and human mammary arteries are used to bypass blocked cardiac arteries. Overall, the survival rate for women is poorer after such operations, however in women who have been pregnant and have breast-fed their offspring, the prognosis is much better, since their mammary arteries are larger with greater blood flow. Crucially, the reasons for these arterial changes are largely unknown, thus a better understanding of how reproductive hormones are involved in the "remodelling" of mammary arteries is not just of academic interest, but may be of use to those involved in such clinical procedures. It is an appropriate time to seek further knowledge about interactions between reproductive hormones and vascular physiology: cardiovascular disease is still a major cause of death in women in the Western world, and scientific debate about the cardiovascular benefits or otherwise of hormone-replacement therapy in peri-/postmenopausal women is still ongoing. Hence clarification of some of the mechanisms linking vascular changes with levels of a major reproductive hormone are timely.
This work is aimed at determining functional and physical changes in mammary arteries from rodents, and how these changes are affected by exposure to the major reproductive hormone in these models. This proposal brings together and can draw on the expertise of staff at Queen Margaret and Strathclyde Universities whose backgrounds in mammary artery physiology, oestrogen biology, cardiovascular and growth factor function give a unique blend of skills which can be applied to this project. The recent investment at Strathclyde in multi-photon technology in the biophotonics suite also allows ready access to world-class facilities which can be applied to the parts of the project dealing with changes in biological structure. This project requires a dedicated experienced researcher at post-doctoral level to arrange, administer, and carry out the practical aspects to the research; in addition, use will be made of existing facilities such as

the excellent animal unit at Strathclyde which is experienced in setting up and maintaining a rodent colony which allows animals at particular stages to be selected in a randomised fashion. It is anticipated that this work will not only advance basic scientific knowledge, but will also provide information about the mechanism of human mammary artery remodelling in women during pregnancy and breast-feeding, which may in turn lead to therapeutic manoeuvres which could pharmacologically remodel these vessels prior to surgery, thus improving the prognosis for women undergoing coronary artery bypass grafting.

Technical Summary (mandatory)

Describe the proposed research in a manner suitable for a specialist reader. This summary will be made publicly available if the proposal is funded. [up to 2000 characters]

The demands of the mammary gland during the reproductive cycle vary from quiescence to peak lactation, & mammary blood flow is greatest at peak lactation. In our current RERAD-sponsored project, we are studying rat mammary artery (RMA) tone during the reproductive cycle & have found that the lowest contractile force was at peak lactation, when blood oestradiol levels are lowest. We also found histological evidence of changes in artery structure, with a decreased vascular muscle/lumen ratio at peak lactation. Oestradiol, the major oestrogen in the rat, may play a role in remodelling mammary vessels during the reproductive cycle: physiological levels of oestradiol increase vascular smooth muscle cell (VSMC) number, inhibit DNA synthesis, & decrease neointimal thickening following injury. This is significant since many other vascular effects of oestradiol such as Ca2+ mobilisation or vessel relaxation require pharmacological levels of the steroid. Many proliferative or hypertrophic agents initially stimulate Na+/H+ exchange (NHE), resulting in cytosolic alkalinisation which correlates with increased DNA synthesis. In addition, cytosolic alkalinisation is associated with increased contraction of vascular muscle. The mechanisms by which oestradiol affects vascular remodelling have not been defined, though cytosolic alkalinisation may play a part since low oestradiol concentrations stimulate NHE activity in VSMC, whereas higher levels inhibit it. We postulate that the ability of oestradiol to modulate VSMC NHE may be pivotal to the anatomical and functional changes occurring in the RMA during reproduction. Thus our aim is to mimic the effects of reproduction by pharmacological manipulation of blood oestradiol in ovariectomised rodents using continuous-release pellets, & compare those responses with animals undergoing normal reproduction. Use of genetically-altered animals will allow us to assess the contribution of classical estrogen receptors.

Beneficiaries (mandatory)

Describe who will benefit from the research [up to 4000 character]

This proposal is aimed at identifying fundamental mechanisms involved in the interaction between oestradiol and the mammary artery during different reproductive states, and it is not anticipated that there will any immediate benefit to end-users in the short-term. However the information gained may be of use to those investigating the role of oestradiol in hormone replacement therapy, or vascular changes related to ageing in women. In addition, the novel animal model used in this proposal is also of potential interest to health care professionals using coronary artery bypass grafting (CABG), since the human internal mammary artery (HIMA) is still the conduit of choice for this technique, and there are clear differences in suitability of this vessel between women who have breast-fed, and those who have not. Information obtained from this proposal can be used to justify similar though non-invasive studies in humans, and how oestrogen status may impact on peri- and postoperative complications or timing of operations when using HIMA. In addition, if hormone-mediated vascular remodelling can be demonstrated, this opens up the possibility of pharmacological interventions in suitable candidates prior to surgery to aid vessel patency and patient prognosis, thus results of the research would be of interest to pharmaceutical and surgical colleagues. As far as we are aware, we are the only group in the UK and possibly elsewhere studying this rodent vessel in this way. Further development and knowledge of our model would make it a useful adjunct to justify research related to the suitability of HIMA in different groups of patients; for example, it has recently been shown that HIMAs from diabetic patients are more responsive to stimulation than nondiabetic patients. Our model could be used to examine the underlying cellular mechanisms for this and, similar situations, thus an in-depth understanding of the normal biology of the artery in an animal model is essential, and may ultimately lead to increased clinical interest in mammary artery physiology. This project will allow the present post-doctoral research fellow to expand his experience from basic myography and histology into cellular ion measurements and fluorescent/confocal imaging in an Institute with world-class facilities and expertise in these areas. For these reasons, this proposal provides an excellent opportunity for the post-doctoral researcher to be at the forefront of research in an exciting and expanding area, and to develop and increase his transferable skills by acquiring expertise in sought-after techniques such as confocal microscopy and digital image analysis. Research by the Cardiovascular Group at Strathclyde is internationally competitive, thus experience within this group and successful completion of this project with the expected publications will place him in an excellent position to develop his own future research, and he will be well-placed to apply for further academic posts and supportive grants in his own right. This will also strengthen the Cardiovascular Group's reputation in this important yet neglected area, and further enhance the standing of the Strathclyde Institute as a centre of excellence in contemporary biomedical research.

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Summary of Resources Required for Project

Financial resources

Summary fund heading	Fund heading	Full economic Cost	BBSRC contribution	% BBSRC contribution
Directly Incurred	Staff	0.00	0.00	80
	Travel & Subsistence	1600.00	1280.00	80
	Equipment	0.00	0.00	80
	Other Costs	200.00	160.00	80
	Sub-total	**1800.00**	**1440.00**	
Directly Allocated	Investigators	11490.00	9192.00	80
	Estates Costs	1210.00	968.00	80
	Other Directly Allocated	0.00	0.00	80
	Sub-total	**12700.00**	**10160.00**	
Indirect Costs	Indirect Costs	5950.00	4760.00	80
	Total	**20450.00**	**16360.00**	

Summary of staff effort requested

	Months
Investigator	2.5
Researcher	0
Technician	0
Other	0
Visiting Researcher	0
Student	0
Total	2.5

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Joint Proposals

Complete this section if more than one organisation is submitting a BBSRC proposal form for this project.

Is this part of a joint proposal ?	☒Yes ☐No
Are you the lead RO ?	☒Yes ☐No
Joint reference	H784805
Total number of proposals	2

Research Councils / MoD Joint Research Grants Scheme (JGS)

If MoD/DSTL have indicated that they are prepared to provide support for this proposal if successful, please indicate the percentage level of this support and MoD/DSTL contact details.

Percentage funding indicated by DSTL	**DSTL contact**	**DSTL Reference** (a letter providing this reference is attached with the Case for Support)

Other Support

Details of support sought or received from any other source for this or other research in the same field.

Awarding Organisation	**Awarding Organisation's Reference**	**Title of project**	**Decision Made (Y/N)**	**Award Made (Y/N)**	**Start Date**	**End Date**	**Amount Sought/Awarded (£)**
Scottish Executive Env and Rural Affairs	2006	Pharmacological & Physiological Characterisation of the Rat Mammary Artery	Y	Y	01/04/2006	31/03/2009	549000

Related Proposals

Proposal is related to a previous proposal to BBSRC

Reference Number	**How related?**

Related Grants

Provide the BBSRC reference numbers of any current grants held by any of the applicants that are related to this proposal. Interim reports of these grants should be submitted with this proposal.	

Staff

Directly Incurred Posts

			EFFORT ON PROJECT							
Role	**Name /Post Identifier**	**Start Date**	**Period on Project (months)**	**% of Full Time**	**Scale**	**Increment Date**	**Basic Starting Salary**	**London Allowance (£)**	**Super-annuation and NI (£)**	**Total cost on grant (£)**
									Total	0

Applicants

Role	Name	Post will outlast project (Y/N)	Contracted working week as a % of full time work	Total number of hours to be **charged** to the grant over the duration of the grant	Average number of hours per week **charged** to the grant	Rate of Salary pool/banding	Cost estimate
Principal Investigator	Dr Iain Gow	Y	100	330	3.8	57450	11490
						Total	11490

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Equipment (the cumulative value of equipment over £50,000 will be treated as an exception)

Description	Country of Manufacture	Delivery Date	Basic price £	Import duty £	VAT £	Total £
					Total £	0

Travel and Subsistence

Destination and purpose		Total £
Within UK	40 site lab vists @ 100 miles per visit	1440
Within UK	Parking costs - 40 x £4	160
	Total £	1600

Other Directly Incurred Costs

Description	Total £
Consumables	200
Total £	200

Other Directly Allocated Costs

Description	Total £
	0
Total £	0

Research Facilities/Existing Equipment

Description	Total £
	0
Total £	0

Animal Costs

Animal Species	Type and Microbiological Quality	Genetically Altered?	No. Purchased	Average Cost per Animal (£)	No. Bred	Average Cost per Animal (£)	Maintenance duration (weeks)	Weekly Maintenance costs per Species (£)	Total Cost £
								Total £	0

Research Council Facilities

Name of Facility	Proposed Usage

Project Partners: details of partners in the project and their contributions to the research. These contributions are in addition to resources identified above.

	Name of partner organisation	Division or Department	Name of contact

Direct contribution to project			Indirect contribution to project		
	Description	Value £		Description	Value £
cash			use of facilities/ equipment		
equipment/materials			staff time		
secondment of staff			other		
other			Sub-Total		
Sub-Total				Total Contribution	

Total Contribution from all Project partners £0

Ethical Information (mandatory)

Please answer the following questions as appropriate

a) Human Participation

Would the project involve the use of human subjects?	Yes☐	No☒
If yes, would equal numbers of males and females be used?	Yes☐	No☒
Would the project involve the use of human tissue?	Yes☐	No☒
Would the project involve the use of biological samples?	Yes☐	No☒
Would the project involve the administration of drugs, chemical agents or vaccines to humans?	Yes☐	No☒
Will personal information be used?	Yes☐	No☒
If yes, will the information be anonymised and unlinked?	Yes☐	No☒
Or will it be anonymised and linked?	Yes☐	No☒
Will the research participants be identifiable?	Yes☐	No☒

b) Animal Research

Would the project involve the use of vertebrate animals or other organisms covered by the Animals (Scientific Procedures) Act?	Yes☒	No☐
If yes, what would be the severity of the procedures?	Mild	☐
	Moderate	☒
	Substantial	☐
Please provide details of any areas of substantial or moderate severity:		
Ovariectomy and implantation of oestradiol pellets by trochar		

c) Genetic and Biological Risk

Would the project involve the production and/or use of genetically modified animals?	Yes☒	No☐
If yes, will genetic modification be used as an experimental tool, e.g., to study the function of a gene in a genetically modified organism?	Yes☒	No☐
And will the research involve the release of genetically modified organisms?	Yes☐	No☒
And will the research be aimed at the ultimate development of commercial or industrial genetically modified products or processes?	Yes☐	No☒
Would the project involve the production and/or use of genetically modified plants?	Yes☐	No☒
If yes, will genetic modification be used as an experimental tool, e.g., to study the function of a gene in a genetically modified organism?	Yes☐	No☒
And will the research involve the release of genetically modified organisms?	Yes☐	No☒
And will the research be aimed at the ultimate development of commercial or industrial genetically modified products or processes?	Yes☐	No☒
Would the project involve the production and/or use of genetically modified microbes?	Yes☐	No☒
If yes, will genetic modification be used as an experimental tool, e.g., to study the function of a gene in a genetically modified organism?	Yes☐	No☒
And will the research involve the release of genetically modified organisms?	Yes☐	No☒
And will the research be aimed at the ultimate development of commercial or industrial genetically modified products or processes?	Yes☐	No☒

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d) Approvals

Have the following necessary approvals been given by:			
The Regional Multicentre Research Ethics Committee (MREC) or Local Research Ethics Committee (LREC)?	Yes☐	No☐	Not required☒
The Human Fertilisation and Embryology Authority?	Yes☐	No☐	Not required☒
The Home Office (in relation to personal and project licences, and certificates of designation)?	Yes☐	No☒	Not required☐
The Gene Therapy Advisory Committee?	Yes☐	No☐	Not required☒
The UK Xenotransplantation Interim Regulatory Authority?	Yes☐	No☐	Not required☒
Administration of Radioactive Substances Advisory Committee (ARSAC)?	Yes☐	No☐	Not required☒
Other bodies as appropriate? Please specify.			

e) Other Issues

Are there any other details of which the Council should be aware?	Yes☐	No☒
If yes, please give details.		

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Classification of Proposal

a Theme and Priority Areas (mandatory)

Indicate the theme and priority area of the committee selected that is relevant to the research proposal.

Theme:	AS: Integrative Animal Physiology (SYS)
Priority Area(s)	
Main (Mandatory):	AS: Genes to Physiology (FGIP)
Secondary 1 (Optional):	AS: Not in a Priority Area

(b) Strategic Plan Objectives (mandatory)

Indicate the strategic plan objectives to which the research proposal would contribute by ticking the appropriate tick-boxes (see definitions in the help file). Please apply one to five classifiers.

Integrative Biology		**Sustainable Agriculture**		**The Healthy Organism**		**Bioscience For Industry**		**Tools And Technology**	
Animal welfare (experimental)		Animal welfare (sustainability)		Ageing (research to understand human ageing)		Biocatalysis		Analytical tools for the biosciences	
Chromosome structure and epigenetics		Control of animal diseases (e.g.vaccinology)		Integrative brain science and behaviour		Biomaterials		Bionanotechnology	
Communication and signalling		Ecology of managed landscapes		Cloning		Bioprocessing		E-science (including bioinformatics)	
Functional and comparative genomics		Host/pathogen interactions - animals		Diet and health in humans, including diet-gene interactions		Environmental biotechnology		Genetic modification	
Genome sequencing		Host/pathogen interactions - plants		Normal (healthy) physiology and development (animals and humans)	x	Automated systems for high throughput		Genomics	
Integrative biology - animals	x	Modelling and epidemiology - animal disease		Stem cells, including cell delivery and survival in transplants		Industrial exploitation of plants and microbes, including secondary metabolism for bioindustry		GRID technology	
Integrative biology - microbes		Modelling and epidemiology - plant disease		Tissue engineering		Microbial food safety		Mathematical models	
Integrative biology - plants		Sustainable agriculture, including precision agriculture		Neuroscience		Unculturable microorganisms for exploitation		Resources for proteomics	
Proteomics, including molecular structure and function determination		Technologies for diagnosis of disease - animals				New pharmaceuticals		Resources for metabolomics	
Transcriptomics		Technologies for diagnosis of disease - plants						Tools for structural biology, including high throughput techniques	
Metabolomics								Resources for transcriptomics	
Whole organism biology	x								

(ii) Keywords (mandatory)

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Keyword	Research Topic	Science Area
Parturition	Animal reproduction	Animal science
Pregnancy	Animal reproduction	Animal science
Cardiovascular systems	Animal and human physiology	Animal science
Integrative mammalian physiology	Animal and human physiology	Animal science
Vessels	Animal and human physiology	Animal science
Reproductive hormones	Endocrinology	Animal science
Steroids	Endocrinology	Animal science
Endocrine receptors	Receptors	Biomolecular science, biochemistry and chemical biology

OTHER INFORMATION

Reviewers (mandatory)

1	Name	Address	Town	Email Address
Professor M Nielson		Department of Anatomy and Physiology	Frederiksberg	mon@kvl.dk
Keywords	Mammary artery, mammary blood flow			

Reviewers (mandatory)

2	Name	Organisation	Division or Department	Email Address
Professor Cherry Lindsey Wainwright		The Robert Gordon University	School of Pharmacy	c.wainwright@rgu.ac.uk
Keywords	cardiovascular disease, cardiovascula pathology			

Reviewers (mandatory)

3	Name	Organisation	Division or Department	Email Address
Professor John McGrath		University of Glasgow	Institute of Biomedical & Life Sciences	I.McGrath@bio.gla.ac.uk
Keywords	cardiovascular disease, adrenoceptors, adventitia, nitric oxide, confocal microscopy			

Reviewers (mandatory)

4	Name	Organisation	Division or Department	Email Address
Dr Alison Douglas		University of Edinburgh	Centre for Integrative Physiology	alison.j.douglas@ed.ac.uk
Keywords	reproduction, lactation, weaning			

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